Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	609	sasai.inv.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/10/30 13:16
L2	12	sasai.inv. and nishikawa.inv.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/10/30 13:16
L3	0	(sasai.inv. and nishikawa.inv.) and ((neural with stem with cell) SAME (RA or retinoic))	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/10/30 13:18
L4	1	(sasai.inv. and nishikawa.inv.) and (((neural or nerve) with stem with cell) SAME (RA or retinoic))	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/10/30 13:22
L5	1	(sasai.inv. and nishikawa.inv.) and (((neural or nerve)) SAME (RA or retinoic))	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/10/30 13:32
L6	1	(sasai.inv. and nishikawa.inv.) and (((neural or nerve)) SAME (RA or retinoic) and aggregation)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/10/30 13:33
L7	1	(sasai.inv. and nishikawa.inv.) and (((neural or nerve)) SAME (RA or retinoic) same aggregation)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2005/10/30 13:33

[0221] In order to induce differentiation of an ectodermal cell or an ectoderm-derived cell into a cell of neural tube or neural crest, the above step using a medium which does not contain BMP4 is carried out and then, when differentiation of the embryonic stem cell into a neuroectoderm is started (e.g., 1 to 14 days, preferably 2 to 8 days, and more preferably 4 to 6 days after starting of culturing), culturing using a medium containing shh or BMP4 is continuously carried out along with optional exchange of the medium.

[0458] Differentiation of embryonic stem cell into non-neuroectodermal cell:

[0459] A medium was produced by adding 0.5 nmol/l BMP4 (manufactured by R & D) to the serum-free medium described in Example 1. The ES cell EB5 was cocultured with PA6 cell according to the method described in Example 1, using the thus produced BMP4-added serum-free medium instead of the serum-free medium used in Example 1. Eight days after culturing, immunological cell staining was carried out using the anti-NCAM antibody, the anti-nestin antibody or an antibody against a non-neural ectoderm cell marker E cadherin (manufactured by Takara Shuzo). As a control, coculturing was carried out using the serum-free medium without BMP4. The results are shown in FIGS. 5A, B, C, D, E and F.

[0460] Also, 8 days after culturing using the BMP4-added serum-free medium, the medium was changed to Glasgow MEM medium containing 10% fetal bovine serum (manufactured by GIBCO BRL), followed by culturing for 3 days. The thus cultured cells were fixed for 30 minutes by adding 4% p-formaldehyde and immunologically stained using an antibody against a skin epidermis cell marker keratin 14 (manufactured by Biomedia), and the results were compared with those in which culturing was continued for additional 3 days using the bovine serum-free medium, with the results shown in FIGS. 5G, R and I.

[0461] As shown in Example 1, when the medium without BMP4 was used, the ES cell-derived colonies were strongly anti-NCAM antibody-positive (FIG. 5A) and strongly anti-nestin antibody-positive (FIG. 5B), whereas the number of E cadherin-positive colonies was small (18%) (FIG. 5C). On the other hand, when the BMP4-added serum-free medium was used, the ES cell-derived colonies were anti-NCAM antibody-negative (FIG. 5D) and anti-nestin antibody-negative (FIG. 5E), whereas E cadherin-positive colonies appeared at a high

frequency (98%)
(FIG. 5F). Keratin 14-positive colonies were not formed when the medium
without no BMP4 was used (FIG. 5G), whereas they appeared when the BMP4-added
serum-free medium was used at a frequency of 34% (FIG. 5H). When culturing was
carried out for 8 days using the BMP4-added serum-free medium and then for next
3 days using the Glasgow MEM medium containing 10% fetal bovine serum, both of
the frequency of keratin 14-positive colonies (47%) and the colony size
significantly increased (FIG. 5I).

[0462] Also, a result similar to the above was obtained when coculturing was
carried out using a typical ES cell, 129 line mouse-derived CCE cell (M. R.
Kuehn et al., Nature, 326, 295 (1987); Production of Mutation Mice Using ES
Cell).